AN OCCURRENCE OF SPIRAL SEGMENTATION IN APANTESIS NAIS (LEP., ARCT.)

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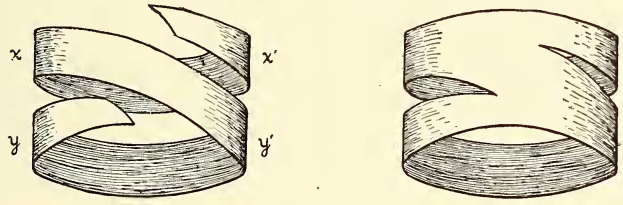
Abnormal segmentation is one of the rarest forms of aberration to be found in insects and among such aberrations, which include fused, doubled, absent, split, incomplete and otherwise malformed somites, one of the most interesting is the condition known as spiral segmentation.

Spiral segmentation is due, apparently, to the fusion of opposite halves of adjacent segments with each other, to the exclusion of their proper halves; and since the faulty union occurs on only one aspect (either dorsal or ventral) and the somites have their normal relation on the opposite aspect, the result is a spiral formation of the involved segments. The condition is



A B

shown diagramatically in the text-figure A, which represents two segments fused on their upper surface (the left half of x being continuous with the right half of y) while on the lower surface they retain their normal relations.

The spiral may be complete, if the half segment which is left without a complement (either dorsally or ventrally) at the beginning and end of the spiral is completely separated from its proper half by a suture, as indicated in fig. A; or incomplete, when the halves remain more or less united, as in fig. B. The spiral may include two or more segments. In most of the cases reported in insects only two segments are involved, but occasionally three, and in one instance six segments are included. Once

established, the condition persists through all subsequent stages in the life of the insect.

Spiral segmentation was first described by Cori, and by Morgan, in papers which appeared at about the same time. Cori observed the condition in earthworms and polychaetes; in certain of the latter it is very common. It is not infrequent in earthworms along with other segmental anomalies, and Morgan's observations on these animals represent the only intensive study of spiral and other segmental anomalies which has been made. Two examples of spiral segmentation have been reported in Myriapods.

In insects spiral segmentation, or other segmental anomaly, is very rarely met with. Arendsen Hein in the course of experiments with the beetle *Tenebrio molitor* observed nearly 50,000 larvæ and among them found 104 which showed various segmental anomalies; some of these were of the spiral type. Abnormal segmentation, including the spiral, is also found in that very variable insect, *Drosophila*. Aside from its occurrence in these two species only seventeen examples of spiral segmentation in insects are described in the literature. Nearly half of these were reported not long ago by Dr. Cockayne; his paper also includes a brief summary of previously described examples, and presents the only critical discussion of the subject of anomalous segmentation in insects which has heretofore been published.

Four of the known instances of spiral segmentation in insects are in Lepidoptera. Christeller first described the condition in this order in a pupa (and its moth) of *Celerio euphorbiæ* in which the fourth to sixth abdominal segments form a spiral. The other three cases were described by Cockayne in the paper just referred to, in larvæ of *Pieris rapæ, Hipocrita jacobaeæ* and *Earias chlorana*. The latter is remarkable in having two separate spirals. In all these larvæ, the spirals involve two abdominal segments: the 2nd and 3d, and the 4th and 5th, respectively in *rapæ* and *jacobaeæ*: and the 3d and 4th, and 7th and 8th, in *chlorana*. The spirals are more or less incomplete in all.

The fact that only four instances of spiral segmentation have been recorded in Lepidoptera lends particular interest to the

case to be reported in which the condition occurred in six larvæ of one brood.

SPIRAL SEGMENTATION IN APANTESIS NAIS

In June of 1929 about forty eggs of *Apantesis nais* (Dru.) were received through the kindness of Mr. C. Rummel, from Green Village, N. J. The larvæ were normal, and the moths emerged in July. Three pairs of these moths were mated and their offspring were designated as broods nos. 114, 115 and 116. When these larvæ were about half grown certain individuals were conspicuous because of an irregularity of the dorsal line. This condition (which in all probability had been present at hatching but passed unnoticed while the larvæ were small) was at first attributed to injury, but on later examination proved to be due to spiral segmentation. The larvæ of all three broods, which numbered from 100 to 150 in each, were then carefully looked over and yielded a total of six abnormal larvæ in brood no. 116, while all the larvæ of the other broods were normal. All were reared under the same conditions.

The larvæ of *nais* hibernate and in the early fall the majority become lethargic and develop slowly, if at all. A small minority, however, will continue to feed actively and complete their metamorphosis without delay. Among eight such individuals of brood 116 were two of the abnormal larvæ, both of which gave female moths.

One of the abnormal females was mated with a male from one of the other broods; the larvæ from this pair were normal, as would be expected. Unfortunately, another generation could not be obtained as all larvæ which hibernated failed to survive the winter; while the moths obtained by forcing larvæ indoors could not be induced to mate, probably because of impaired vitality due to the unnatural conditions under which they were reared. The same difficulty has been frequently encountered in this and other species which have been forced through the winter.

The larvæ of *nais* are black, often (as in this lot) with a conspicuous yellowish dorsal line, or stripe; and with black warts bearing many setæ, of which wart i is very small, the rest quite large.

The conspicuous feature of the abnormal larvæ is the oblique displacement of the dorsal line over the involved segments, with a small offshoot at each angle running to the end of the incomplete suture.

The segmental abnormality is essentially the same in all six larvæ being of the spiral type with the involved segments incompletely separated and affecting either the 4th and 5th, or the 5th and 6th abdominal somites. The spiral is right handed in some, left handed in others. The defect in segmentation is evident only on the dorsum; the ventral surface appears normal.

All the warts are present, normal in size, with the usual setæ; spiracles are all present. With the exception of wart i, which is often crowded out of its normal relation to ii, all the warts are in practically normal relation to each other in each half-segment. Where there is marked outward displacement of a half-segment (*viz.*, right half of segment 4, left half of 5, in Fig. 2, Plate I) it is evident on lateral view by a lower position of the warts relative to those of the adjacent segments; this is due to the displacement of the half-segment as a unit, however, and not to any change in the normal relative positions of its warts. The larvæ were of normal size and vigor.

The appearance of the involved segments strongly suggests an effort on the part of the organism to form a single perfect segment by the oblique union of two half-segments at the expense of their proper halves. In Fig. 2 (Plate I), for instance, the left half of segment 5 and right half of segment 4 are crowded outward and narrowed, while left 4 and right 5 unite to form an oblique tergite of nearly normal width. Moreover, the warts of the half segments forming this oblique union tend to assume an arrangement which is normal relative to the abnormal segment as a whole. This is achieved to a remarkable extent in the larvæ shown in Fig. 2, in which the warts of the oblique segment have exactly the same relation to each other and to the obliquely displaced dorsal line as they would have on a normal segment.

The combined length of the two spiral segments on the dorsum is somewhat less than that of two normal ones, but the half-segments which are crowded out and narrowed above broaden to normal width below.

Each of the larvæ had the appearance of being slightly twisted on its longitudinal axis at the point of abnormality, the dorsal line and warts of the normal segments anterior to the spiral being distinctly out of line with those of the segments posterior to it. This appearance of torsion was much more marked in some larvæ than in others. It is evident in the segments shown in the drawings, and in the living larva seen as a whole was further emphasized by a little difference in the alignment of the spiracles and lateral warts of the anterior and posterior parts of the body. When some of these larvæ were crawling it appeared as if the posterior part did not "follow through" accurately, though the ventral surface of the larva was normal and there was no apparent displacement of the prolegs.

Figures 3 and 4 show the pupa-skins of the two moths which were reared. The caterpillars which formed these pupæ were essentially similar to the ones figured (except that in the insect shown in Fig. 3, the 5th and 6th segments were involved, instead of the 4th and 5th.) It is noticeable that while the abnormal segments are not completely separated in the larvæ, they have become distinctly separated in one of the pupæ (Fig. 3, in which the spiral effect is unusually clear) and nearly so in the other. Aside from the spiral segmentation the pupæ were normal.

The moths showed the same defect, and one of them was abnormal in coloration in that the markings of the fore-wings, normally yellow, were obsolescent on the distal half of the wing and dull brownish in color.

The Cause and Origin of Abnormal Segmentation

The cause of segmental abnormality, of which spiral segmentation is one of the manifestations, is not known. While the evidence bearing on the occurrence of segmental anomalies is too scanty to warrant final conclusions as to their cause, it may nevertheless be interesting to review such facts and theories as are at hand.

It has often been supposed that abnormalities of segmentation were due to injuries received in the larval or pupal state. Possibly some defects are so caused, but such an injury as might conceivably produce so extensive a defect as spiral segmentation, for instance, would certainly result in the death of

the insect. There can be no doubt that most segmental anomalies, if not all, are developmental in origin.

Morgan found that in worms segmental anomalies often originated in the embryo. It might be expected that in insects also such abnormalities would be found to originate in the egg stage, and this supposition is confirmed by the finding of well established segmental defects in larvæ, and by the finding of embryos with such defects by Cappe de Baillon.

In explanation of the origin of segmental anomalies in worms, Morgan suggested that they were due to the unequal or irregular growth of the blocks of tissue which appear on each side of the mid-line in the embryo and represent primitive half-somites; that as a result of such irregular growth a half-somite would fail to unite evenly with its proper opposite half, but instead would overlap and unite with the half-somite adjacent thereto. If the overlapping occurred on only one aspect, a spiral would result. He made no statement as to what might be the cause of such irregular growth, apparently regarding it as due to some developmental peculiarity inherent in the individual.

A very similar theory was offered by Cori, who further suggested that the cause might lie in an unusually favorable environment during development, the abnormality resulting from unduly rapid growth.

Chapman, speaking particularly of spiral segmentation, thought the condition arose during the period of dorsal closure of the body cavity, and was due to the failure of the half-somites of one side to meet their corresponding opposites.

Spiral as well as other defects may be ventral, however, and Cockayne points out that Chapman's theory would not account for these; referring to instances where both dorsal and ventral defects occur in the same individual and assuming that all segmental defects are probably of similar origin, he argues that since ventral defects must originate very early in the process of segmentation in the embryo, then all segmental defects must arise in a "primary error" of segmentation.

Cappe de Baillon, in the course of his work on double monsters of the phasmid *Carausius morosus,* found segmental abnormality relatively frequent in embryos of such monsters, but regarded it as a purely secondary phenomenom resulting from pressure

within the egg caused by the unusually large embryo. "Selon toute vraisemblance," he says, "il s'agit ici d'une malformation banale qui n'a rien à voir avec la monstruosité double. Elle est simplement la conséquence du volume exagéré de l'embryon qui remplit déjà l'oeuf, alors que son abdomen est encore à peine formé." These segmental defects were not incompatible with life, the insects often reaching the adult stage.

The question of inheritance of segmental abnormality was studied by Arendsen Hein. In the course of experiments with *Tenebrio molitor,* previously mentioned, he bred the off-spring of abnormal beetles through several generations without increase in the incidence of abnormality over that in unselected stock, and concluded that segemental anomalies were not inherited.

In the fruit fly, however, inheritance evidently does take place. According to Morgan (who made the observations in the course of genetic studies without particular regard to the question of segmentation) individuals with irregularities of the rings of the abdomen are not uncommon in *Drosophila,* and he says, "sometimes they appear to have been caused by injury to the larvæ or pupæ, but still other abnormalities are inherited in the sense that they occur in certain stocks in more less definite percentages."

One race in which extreme irregularity of segmentation occurred, described by Bridges and called "patched," came from a single female with reduced number (three) of segments.

The abnormal condition in flies of this sort was not influenced by environment, and the comparative frequency with which it appeared in inbred stock, and its rarity when the stock was outcrossed, was evidence of its inherent nature. Morgan was unable to secure a pure stock of such abnormals, however, the cultures invariably throwing a very high percentage of normals.

The occurrence of the six abnormal *nais* larvæ in one brood while sister broods were normal similarly suggests a genetic basis for the aberration.

Some comment may be offered on the facts presented in the foregoing review, without attempting to reach conclusions. As the evidence stands, the inference is that abnormal segmentation may result from different causes; on the one hand inherent and primary, on the other external and secondary; such a varied

origin would imply a corresponding variation in the extent of the structural involvement.

In regard to the question of primary developmental origin, it may be remarked that Morgan found that internal structures in earthworms corresponded with the external segmental defects, indicating a fundamental fault in the process of segmentation. While worms and insects are hardly comparable structurally, still it is reasonable to suppose that in the latter also such anomalies may be of similar origin, (or in other words are due to a "primary error" of development) and that the segmental involvement is similarly deep-seated in extent.

Such an early and fundamental error of development might well be expected to originate in a germinal abnormality inherent in the individual, and such an origin is clearly indicated by the inheritance of the condition in *Drosophila;* in such a case the cause must lie in some disturbance of the remote and inexplicable forces which govern the development of an organism.

On the other hand, the observations of Cappe de Baillon lead to an exactly opposite hypothesis. It is obvious that one must proceed with reservation in attempting to draw a parallel between anomalies occurring in the composite eggs of double monsters and those of normal insects. The basic conditions of development are the same in both, however, and justify consideration of Cappe de Baillon's observations,— which indicate that segmental anomaly may be an entirely secondary phenomenon in no way inherent but brought about by some extraneous cause.

From this point of view, abnormal segmentation might have its inception either early or late in the course of development, depending on what the cause might be and its time of action, and the extent and location of the structural involvement would vary accordingly. Chapman's theory of a fault in the dorsal closure may be recalled in this connection; a theory which receives support in the great preponderance of dorsal over ventral defects, particularly in the case of spirals.

Cockayne is inclined to believe that most segmental anomalies "taken at large are not inherent and must be due to some external cause acting upon the ovum at the very early stage when the somites are being formed."

His opinion, however, is largely based on the negative results of Arendsen Hein with regard to inheritance in *T. molitor,* and upon a condition of segmental anomaly in *Drosophila* showing sex-linked inheritance which will be considered later, and which should not be regarded as of the same type of anomaly of embryonic origin as that now under discussion. Cockayne refers to Cappe de Baillon's observations but, perhaps correctly, does not consider them applicable to examples of segmental anomaly in general.

Nevertheless, it may be tentatively remarked that possibly in the normal egg the embryo may rarely suffer some displacement or malposition which results in unequal pressure on developing segments. This would bring about some such unequal or irregular growth of the half somites as Morgan suggested, or at a later period interfere with normal dorsal closure. To speculate further on this line, it may be that the torsion of the body in the *nais* larvae and in other examples of spiral segmentation is not, as it obviously appears to be, a result of the deformity, but rather an indication of the cause of it, itself originating in some distortion of the embryo which secondarily resulted in the abnormal segmentation.

In such a case the inherited condition need not be an actual germinal defect, but some instability of the developmental processes which renders certain individuals more liable to developmental accidents, from whatever cause.

So far the consideration of segmental abnormality has been concerned with the appearance of the anomaly during the development of the embryo. Segmental abnormality may also make its appearance during post-embryonic development, however.

A race of *Drosophila* called "abnormal abdomen" and characterized by abnormal arrangement of the bands of the abdomen was reported by Morgan, which was entirely distinct from and not to be confused with those flies mentioned above in which the segmental abnormality appeared sporadically in various stocks. In the race now under consideration the abnormal character showed Mendelian inheritance, behaving as a sex-linked dominant, but appearing only under certain peculiar conditions of environment.

If these flies were reared in a wet culture the defect appeared; as the culture became dry, or if the parents were transferred to a dry bottle, the flies issuing thereafter appeared normal, but their descendants even after several normal generations would again show the abnormality if reared under the essential cultural conditions.

No observations on the larvae are recorded, but since it is stated that the influence of the wet condition did not appear unless the insects were subjected to it through part of the larval life, it must be inferred that the larvæ were normal. In this case the segmental abnormality must make its appearance in the course of the changes which take place during transformation from larva to adult.

A segmental anomaly which appears only in the adult cannot be the result of an error of segmentation in the embryo for in the latter case it would be bound to persist through all stages regardless of external conditions; neither is it probable that normal segmentation once established, and exhibited in the larva, could undergo any fundamental structural alteration in later life. As compared with those originating in a fault of embryonic development, these late appearing anomalies must therefore be comparatively superficial.

It is not improbable that in some anomalies (and this would apply particularly to the late appearing ones) the segmental involvement is essentially external, having its inception in defective development of the new hypoderm which replaces that of the larva during transformation to form the body-wall of the imago. The cause of the defective hypodermal development might ultimately lie in an inherent abnormality of the ectoderm (whether or not influenced by environment, as in *Drosophila*); or it might be due to the effect of external causes alone, as will be mentioned below.

According to this hypothesis anomalies of post-embryonic origin belong in a very different category from those arising in the egg. The only ground for a common origin of both the early and late appearing anomalies would be that the former are also essentially merely ectodermal in origin; and while it is perfectly possible that some of them are, it is more likely, as has already been observed from analogy with worms and for other reasons,

that anomalies of embryonic origin represent fundamental and deep-seated structural aberrations.

It is appropriate to mention here that evidence of ectodermal, or hypodermal abnormality, indicated by defective pigmentation and setae, is not infrequent in insects with segmental aberrations. Many segmental anomalies, too, have an obvious appearance of being due simply to incompletion or malformation of the intersegmental sutures, which suggests a superficial defect. Incidentally, defective pigmentation was particularly mentioned in the description of the "abnormal abdomen" race of *Drosophila*. Defects of pigmentation and setae would be expected in segmental anomaly due to ectodermal abnormality, or to defect of the hypodermal tissues from any other cause.

It is not intended to imply, however, that pigmental and setal defects are a criterion of the origin of the segmental anomalies with which they may be associated, for segmental abnormality due to a primary developmental error might be accompanied by ectodermal abnormality as a secondary condition. It may be noted in this regard that it was occasionally found in worms that the internal and external defects did not correspond, which led Morgan to conclude that at times the ectoderm may vary independently of the mesoderm.

Cockayne has remarked upon abnormality of ectodermal structures accompanying segmental anomaly, and one of his larvae with spiral segmentation (*E. chlorana*) shows extreme abnormality of setal and pigmental structure. On the other hand it will be recalled that in the *nais* larvae there was remarkable integrity of the ectodermal structures, which were in themselves quite normal though more or less displaced by the deformity. Evidently ectodermal abnormality is a secondary condition as regards spiral segmentation.

It is probable that the anomalies which arise in different stages of development are quite distinct in type. This conclusion is supported by the existence of two genotypes in *Drosophila*, in one of which the abnormality depends on a factor in the X-chromosome while in the other it is apparently due to multiple factors. If one may judge from Morgan's and Bridge's figures, too, the segmental defects in the "abnormal abdomen" mutant were much less extensive than in the "patched" variety. Among the

figures of the latter, incidentally, is one showing an excellent spiral.

There remain to be mentioned those abnormalities produced by certain physical agents.

Geigy reported that ultra-violet radiation of the eggs of *Drosophila* produced abnormalities in the adults among which were segmental anomalies (rings irregularly disposed, interrupted, or even missing), although the larvae which came directly from such eggs were of normal appearance.

A similar observation was made by Timofeef-Resovsky, who in the course of certain experiments with X-rays noted incidentally that eggs and larvae subjected to radiation often produced adults with abnormalities of segmentation.

The defects produced by these physical agencies are probably due simply to tissue injury. In the radiation of a very early egg stage the injurious effect may be exerted upon the ectoderm with consequent impairment of ectodermal structures in later life, while in more advanced stages direct injury may be done to the histoblasts from which the adult hypoderm is derived

It may properly be questioned whether some of the abnormalities last considered are to be regarded as segmental anomalies at all, strictly speaking. Preliminary experiments with ultra-violet radiation (suggested by Geigy's paper) which I have made on *Drosophila* indicate that in some cases, at least, the anomalies so produced are entirely superficial without disturbance of segmentation, the appearance of irregularity being due solely to defective chitinization and pigmentation.

The foregoing review of the facts and theories bearing on the cause of abnormal segmentation is intended simply as a résumé of the information at present available in regard to these anomalies. Such comment as has been offered is necessarily largely speculative. Nothing whatever has been recorded on the internal anatomy of segmentally aberrant insects. Most of the observations are concerned with a single extremely variable insect, the fruit fly; and in this insect, with one exception, there is no definite knowledge of the presence or absence of anomalies in the early stages. Neither have segmental anomalies appearing under different conditions, and in different stages, been carefully compared with one another.

We can say only that abnormal segmentation is developmental in origin, and that apparently it may arise in the embryo either as a primary error of segmentation, the cause of which is inherent, or as a secondary result of some extraneous condition. In a different category are segmental anomalies which arise during transformation and appear only in the imago; the involvement in this type must be comparatively superficial as compared with anomalies of the embryonic origin. Still other abnormalities, related to the last type in their more superficial nature, may result from the injurious action of physical agencies. No definite conclusions in regard to the cause and origin of abnormal segmentation will be warranted, however, until much more has been learned about these interesting aberrations.

LITERATURE CITED

ARENDSEN HEIN, S. A., Studies on variation in the meal worm, *Tenebrio molitor*, Jour. Genetics, vol. 10, pp. 227–231, 1920.

BRIDGES, C. B., and MORGAN, T. H., The second-chromosome group of mutant characters, Publ. 278, Carnegie Inst. Wash., 1919; p. 241; pl. 11.

CAPPE DE BAILLON, P., Recherches sur la teratologie des insectes, Encyc. Ent., Ser. A, p. 78, pp. 248–251, 1927.

CHAPMAN, T. A., On insect teratology, Proc. So. Lond. Ent. & N. H. Soc. 1910–11, pp. 39–53.

CHRISTELLER, E., Die missbildungen der schmetterlinge, Ent. Mitt., vol. 6, pp. 14–16, 1917.

COCKAYNE, E. A., Spiral and other anomalous forms of segmentation, Trans. Ent. Soc. Lond., vol. 77, pp. 177–184, 1929.

CORI, C. J., Uber anomalien der segmentirung bei anneliden und deren bedeutung fur die theorie der metamerie, Zs. wiss. Zool., vol. 54, pp. 569–578, 1892.

GEIGY, R., Production d'anomalies par exposition de Drosophiles aux rayons ultre-violets, Verh. schweiz. naturf. Ges., vol. 108, pp. 191–192, 1927.

MORGAN, T. H., Spiral modification of metamerism, Jour. Morphol., vol. 7, pp. 245–251, 1892.

A study of metamerism, Quar. Jour. Micr. Sci., vol. 37, pp. 395–476, 1895.

The rôle of the environment in the realization of a sex-linked Mendelian character in Drosophila, Am. Nat., vol. 49, pp. 385–429, 1915.

TIMOFEEFF-RESSOVSKY, N. W., The effect of X-rays in producing somatic genovariations of a definite locus in different directions in *Drosophila melanogaster*, Am. Nat., vol, 63, p. 122, 1929.

PLATE I

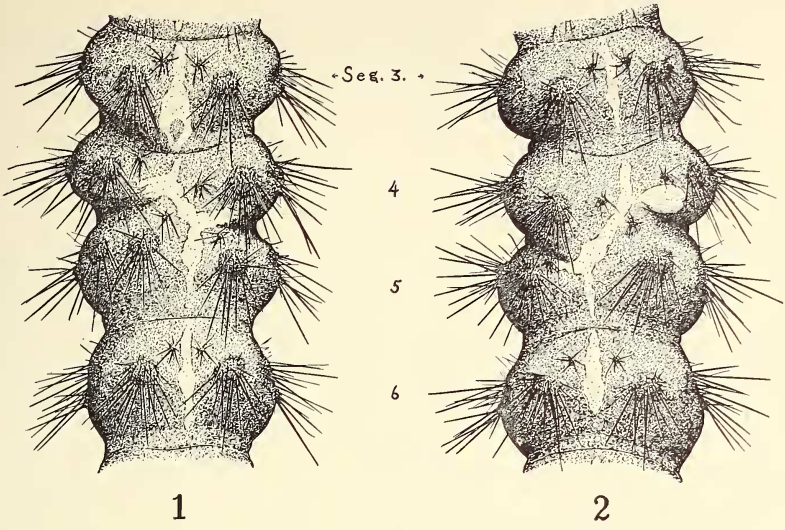
Figure 1. Dorsal aspect of 3rd, 4th, 5th and 6th abdominal segments of a larva of *Apantesis nais* showing spiral segmentation.

Figure 2. Another larva, with the spiral in the opposite direction; the same segments are involved.

Figure 3. Pupal exuvia showing spiral segmentation. The larva which formed this pupa was similar to the one shown in Figure 1, except that the 5th and 6th segments were involved in the spiral instead of the 4th and 5th.

Figure 4. Pupal exuvia. This pupa was formed by a larva similar to Figure 2.

Figure 5. Abdomen of moth which emerged from the pupa shown in Figure 4.



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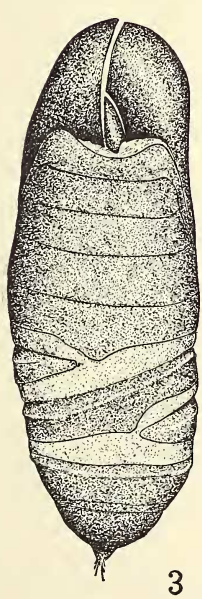

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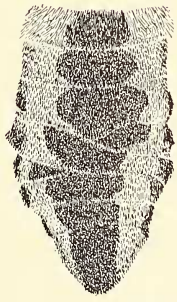

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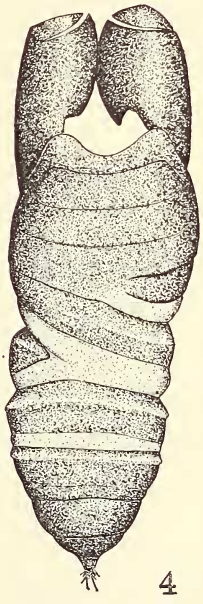

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